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EXAMINER	
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ART UNIT	PAPER NUMBER
1652	15

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/724,296	Applicant(s) DOETSCH ET AL.	
	Examiner Malgorzata A. Walicka	Art Unit 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 March 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *21-25* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *21-25* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *See Continuation Sheet* .

Continuation of Attachment(s) 6). Other: sequence alignment, copies of papers used in 102 and 103 rejection.

The Amendment under 37 CRF 1.111 filed on March 20, 2003 as paper No. 14, is acknowledged. The amendments to the specification and claims have been entered as requested. Claims 16-20 are cancelled. New claims 21-25 are entered. Claims 21-25 are pending in the application and are the subject of this Office Action.

Office Action

1. Objections

A claim 21 is objected to because of the typographical errors. Line 8 contains unnecessary "an"; line 10 contains the abbreviation "UveP1" which should be Uve1p.

2. Rejections

2.1. Lack of utility, 35 USC section 101

Rejection of claims 16-20 made in the previous Office Action, paper No. 12 is withdrawn because the asserted utility SEQ ID NO: 26, 37, 38 and 39, is credible in the light of Exhibit A included in Applicant's response and the fact the SEQ ID NO: 38 is identical to the human MED1 endonuclease of Bellacosa et al; see the rejection under 35 USC section 102 below.

2.2. 35 USC section 102

Claims 21, 22 and 24 are rejected under 35 U.S.C. 102(b) as being anticipated by Takao et al. (Nucleic Acid Res. **1996**, 24, 1267-1271). The claims are directed to the method for cleavage of a double–stranded DNA molecule containing a distorted

structure when the cleavage enzyme is set forth by SEQ ID NO: 4; see the reasons stated in rejection of claims 16, 18, and 20 in the previous Office Actions, paper No. 9 and 12.

Takao et al. cloned *S. pombe* UVDE endonuclease gene consisting of 599 amino acids. Takao et al. showed that the protein truncated up to 229 amino acid from the N-terminus (page 1270 Figure 5 B) retains enzymatic activity towards UV irradiated DNA. SEQ ID NO: 4 of the instant application, called by Applicants "delta 228 variant" is identical to the truncated form of the enzyme consisting of amino acids 229-599 as shown in Figure 5 B of the prior art. Takao et al. also teach on page 1268 the incision assay for their UVDE endonuclease. Thus, claims 21, 22 and 24 are rejected under 35 USC, section 102(b) because SEQ ID NO:4 and the claimed method of its use are anticipated by Takao et al.

In their current response, page 5, line 15, Applicants repeat their previous traverse of paper No.11:

> "this reference teaches that the truncated endonuclease was not stable in pure form, and the assays were carried out with endonuclease preparations which were only about 35% pure. See page 1269, column 1. By contrast, the present application teaches that the truncated UVDE proteins were purified to apparent electrophoretic homogeneity and that proteins made were stable in pure form".

Applicants' arguments have been fully considered, but they are found not persuasive. Takao et al. cloned the gene of *S. pombe* UVDE endonuclease of which amino acids 229-599 are identical to SEQ ID NO: 4 of the instant application. Takao et al. also showed that truncation of the endonuclease up to 232 amino acid from the N-terminus does not influence the endolytic activity of the enzyme on UV irradiated DNA. Takao et al. expressed the protein consisting of amino acid 230-599 in *E. coli* and further used the truncated protein for incision of the UV irradiated DNA. Takao et al. experienced difficulties with purification of the yeast protein expressed in *E. coli*, whereas Applicants, who used as a host *S. cerevisiae* and expression of the truncated UVDE gene in frame with a glutathione-S-transferase leader sequence, a method assuring the expression of a stable protein, were successful in purification of the expressed protein to apparent electrophoretic homogeneity.

In conclusion, Takao at al. used the same method and the same enzyme as claimed in claims 21, 22 and 24 of the instant application. Takao et al. produce the enzyme in E. coli host cell comprising the gene encoding full enzyme or truncated enzyme consisting of amino acids 230-599. The purification of the enzyme from E. coli used by Takao did not result in 100% purity, nevertheless as Applicants' claims are not limited to use of an enzyme purified to homogeneity, the fact is that the enzyme (product) and the method of use of said product were taught by Takao et al. two years before Applicants filled, on June 8, 1998, the provisional application No. 60/088521, of which the instant application claims benefit.

Claim 25 is rejected under 35 U.S.C. 102(b) as being anticipated by Yajima et al. (The EMBO Journal **1995**, 14, 2393-2399.

The claim is directed to the method for cleavage of a double–stranded DNA molecule containing a distorted structure when the cleavage enzyme has the amino acid sequence of SEQ ID NO: 36.

SEQ ID NO: 36 is shown in Figure 2, page 2394 of the paper by Yajima et al. and two versions of incision method are presented on page 2399.

In their response, regarding rejection of claims 16, 18 and 20 under 102 (b), Applicants write on page 6: "The first incision assay taught at page 2399 is one in which closed circular plasmid DNA has been UV-irradiated. The second assay is in which oligonucleotides with pyrimidine dimers have been UV-irradiated [these are dipirimidine sites and not pyrimidine dimers. Pyrimidine dimers are photochemical products resulting from UV irradiation of dipyrimidine sites; emphasis MW]."

Applicants' arguments have been fully considered but are found not persuasive. Yajima et al. cloned the gene encoding the UV specific endonuclease of *Neurospora crassa* having the amino acid sequence identical to SEQ ID NO: 36 of the instant application. In the first assay taught on page 2399 Yajima et al. use, indeed, a closed circular plasmid. However this plasmid was linearized before UV irradiation. Furthermore Applicants 'claims do not exclude use of closed circular plasmid. In the second assay, on the same page, they use an oligonucleotide that was synthesized to specifically contain two dipirymidine sites, which after irradiation form pyrimidine dimer. The oligonucleotide containing the dimer is used as substrate by the enzyme. This

oligonucleotide was paired with complementary oligonucleotide before irradiation. Thus, two stranded piece of DNA was irradiated and subsequently cleaved by the enzyme of amino acid sequence identical to SEQ ID NO: 36. Fig. 7 and 8, page 2397, summarize results of Yajima et al. research.

In conclusion, Yajima et al. teach the product, i.e. the enzyme of SEQ ID NO: 36 and its use in the method for cleavage of damaged double stranded DNA three years before Applicants filed, on June 8, 1998, the provisional application No. 60/088521, of which the instant application claims benefit.

Claim 25 is additionally rejected under 35 U.S.C. 102(b) as being anticipated by PCT Publication WO 99/04626 (WO) issued on February 4 1999; inventor/applicant Bellacosa A. et al.

The claim is directed to the use of the DNA damage nuclease of SEQ ID NO: 38 in a method for cleavage of a double–stranded DNA molecule characterized by a distorted structure.

Bellacosa et al. cloned, sequenced and characterized human MED1 endonuclease having amino acid sequence identical to that of SEQ ID NO: 38, see Fig. 3; sequence alignment enclosed. On page 52 Bellacosa teach endonucleolytic activity assay in which they use calf thymus heat damaged, i.e., denatured double stranded DNA, as a substrate; results of the assay are shown in Fig. 9. In Fig. 12 Bellacosa et al. propose a mechanism of action of the MED1 endonuclease on double stranded DNA; copy of Fig. 12; is enclosed. The skilled artisan concludes that MED1 is of use for

cleavage of distorted double stranded DNA, especially next to uracil originating from demethylated cytosine.

2.3. 35 USC section 103

Claims 21, 23 and 24 are rejected under 35 U.S.C. 102(b) as being anticipated by Takao et al. (Nucleic Acid Res. **1996**, 24, 1267-1271) in view of Ford et al. (Fusion Tails for the Recovery and Purification of Recombinant Proteins, Protein expression and purification, **1991**, 2, 95-107.

The claims are directed to the method for cleavage of a double–stranded DNA molecule containing a distorted structure when the cleavage enzyme is set forth by SEQ ID NO: 2 consisting of glutathione-S-transferase leader sequence, amino acid residues 1-229 and S. pombe UVDE endonuclease, amino acids residues 230-828.

Takao et al. cloned *S. pombe* UVDE endonuclease gene consisting of 599 amino acids, identical to amino acids 230-828 of SEQ ID NO: 2 of the instant application. Takao et al. teach the method of incision of double stranded damaged DNA using their UVDE endonuclease on page 1268, left column; see subtitles *Plasmid nicking assay* and *Incision assay using synthetic oligonucleotides*. Takao et al. expressed the enzyme in *E. coli* and purified it using as the first step heparin-Sepharose column and subsequently blue-Sepharose, page 1269, left column, line 11. However, they experienced difficulties in purification of a stable protein from *E. coli.* Takao et al. also reported successful expression of UVDE gene in S. cerevisiae; page 1271, right column, line 20.

, Takao et al. do no teach, however, how to efficiently recover and purify the UVDE enzyme expressed in any microorganism.

Ford et al. teach that making a fusion protein consisting of glutathione-S-transferase tail (GST) and an enzyme of interest, page 96, right column, line 29, enables efficient recovery and purification using the affinity column containing immobilized glutathione The GST can be subsequently cleaved out of the enzyme by thrombin, if the fusion protein is not active.

It would have been obvious to one having ordinary skill in the art at the time of invention to have the method of DNA cleavage of Takao et al. and to modify the expression and purification of UVDE endonuclease as taught by Ford.

The motivation for the modification would be to have a large quantity of pure enzyme necessary for the method. The motivation is provide by Ford et al. who state, "On a lab scale, fusion tail recovery systems are powerful and elegant tools for one – step recovery and purification of recombinant proteins or identification of proteins encoded by cloned cDNAs. On an industrial scale, fusion tail technology can be used in the recovery and purification of both higher-cost pharmaceuticals and lower-to medium –cost enzymes."

The expectation of success is high because of well-developed and routine use of the glutathion-S-transferase fusion protein in the art.

Thus, the claimed invention was within the ordinary skill in the art to make and use at the time it was made, and was as a whole *prima facie* obvious.

3. Conclusion

No claim is in conditions for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Malgorzata A. Walicka, Ph.D., whose telephone number is (703) 305-7270. The examiner can normally be reached Monday-Friday from 10:00 a.m. to 4:30 p.m.

If attempts to reach examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, Ph.D. can be reached on (703) 308-3804. The fax phone number for this Group is (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionists whose telephone number is (703) 308-0196.

Malgorzata A. Walicka, Ph.D.

Art Unit 1652

Assistant Patent Examiner

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